Testing for *in vitro* genetic toxicity in high dimensional nanomaterial dose-response experiments

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Methodology

The aim is to evaluate nanomaterials toxicity with an underlying assumption that the true dose-response relationship between the dose (concentration) and the response is monotone. Suppose that DNA strand breaks is the endpoint of interest that indicates toxicity (the response).

1. One-Way ANOVA under a simple order restriction.

Suppose that the response variable is denoted by Y and the concentration level by X_i, $i = 0, \ldots, K$ with X_0 being a control dose. Assume that there are in total $K+1$ discrete concentrations, with $X_0 \leq X_1 \leq ... \leq X_K$ and n_i number of observations at concentration X_i. For a specific nanomaterial, the observed data can then be written in pairs as (x_i, y_{ij}), m=1,...,M. Let $\mu(x_i)$ be the mean at the ith dose level. Our aim is to test the homogeneity of the means against an order-restricted alternative. We consider a nanomaterial specific one way ANOVA model of the form.

$$Y_{ijm} = \mu(x_i) + \varepsilon_{ij}. \tag{1}$$

Y_{ijm} denotes the DNA damage of the jth replicate in the ith dose level for the mth nanomaterial. The null hypothesis of no dose effect and the alternative hypothesis of positive dose effect (monotonically increasing means) are formulated as follows:

$$\begin{aligned} H_0 &: \mu(x_0) = \mu(x_1) = ... = \mu(x_K), \\ H_1 &: \mu(x_0) \leq \mu(x_1) \leq ... \leq \mu(x_K). \end{aligned} \tag{2}$$

Note that according to the type of the toxicity endpoint measured, the direction of the ordered mean is assumed to be known. For a decreasing dose-response trend the alternative hypothesis is given by

$$H_1 : \mu(x_0) \geq \mu(x_1) \geq ... \geq \mu(x_K). \tag{3}$$

2. Inference for order restricted alternative

There are several methods that can be applied to test the monotonic trend of the means (Lin et al., 2012). The methods of Williams, and of Marcus, are t-tests for order restricted inference, and are discussed in Section 2.1 while the likelihood ratio test is discussed in Section 2.2.

2.1 Williams and Marcus

For an experiment with n replicates of observation at each dose level, Williams test statistics (Williams, D. A., 1971) is given by:

$$t_i = \frac{\hat{\mu}^\star(x_i) - \bar{y}_0}{\sqrt{2s^2/n}}. \tag{4}$$

with $\hat{\mu}^\star(x_i)$ denotes the estimate of the isotonic mean at dose i, $\bar{y}_0$ the sample mean at dose 0 and s^2 the estimate of the variance (Lin et al., 2012). The isotonic mean at each dose level $\hat{\mu}^\star(x_i)$ are used in the step

down procedure to determine the lowest dose for which a dose effect is detected (Williams, D. A., 1971). In case the number of observations is not equal at each dose level, the test statistics can be modified as follows:

$$t_i = \frac{\hat{\mu}^\star(x_i) - \bar{y}_0}{\sqrt{s^2/n_i + s^2/n_0}}. \tag{5}$$

Marcus modified Williams test statistics, by replacing $\bar{y}_0$ with the mean estimate at dose 0 under order restriction, $\hat{\mu}^\star(x_0)$ (Lin et al., 2012). Thus, the test statistics becomes

$$t_i = \frac{\hat{\mu}^\star(x_i) - \hat{\mu}^\star(x_0)}{\sqrt{s^2/n_i + s^2/n_0}}. \tag{6}$$

2.2 Likelihood ratio test

Another method that can be used to test the equality of the mean response under order restriction is through a Likelihood ratio test. This test can be used to detect a monotone trend but cannot give an indication in which dose(s) there is a difference. The test statistics is given by:

$$\Lambda_{01}^{\frac{2}{N}} = \frac{\hat{\sigma}^2_{H_1}}{\hat{\sigma}^2_{H_0}} = \frac{\sum_{ij}(y_{ijm} - \hat{\mu}_i^\star)^2}{\sum_{ij}(y_{ijm} - \hat{\mu})^2}, \tag{7}$$

with $\hat{\mu} = \sum_{ij} y_{ij} / \sum_i n_i$. It is the ratio between error variance under H_0 and error variance under H_1. The test statistics can also be written in term of

$$\bar{E}^2_{01} = 1 - \Lambda_{01}^{\frac{2}{N}} \tag{8}$$

The null hypothesis is rejected for a large value of $\bar{E}^2_{01}$ or for a small value of $\Lambda_{01}^{\frac{2}{N}}$. When the direction of the trend is unknown, the more likely direction can be chosen by comparing likelihood of the increasing and decreasing trend, the direction with the higher likelihood is selected.

2.3 Inference for high dimensional dose-response experiments

When there is a large number of nanomaterials for which the test for monotone trend is applied, adjustment for multiplicity should be considered. Resampling based inference are used since the sample size of the experiments can be small and the assumption of the distribution of the response might not be fulfilled (Lin et al., 2012). P-values can then be obtained through permutations, by recalculating the test statistics in each permutation. Suppose that permutation matrix $\mathbf{T}$ is

$$\mathbf{T} = \begin{pmatrix} t_{11} & t_{12} & ... & t_{1B} \\ t_{21} & t_{22} & ... & t_{2B} \\ \vdots & \vdots & \vdots & \vdots \\ t_{m1} & t_{m2} & ... & t_{mB} \end{pmatrix}, \tag{9}$$

with t_{mb} as the test statistics for the mth nanomaterial in the bth permutation b. The total number of the permutations is denoted by B. Let t_m be the test statistic for the mth nanomaterials, the row p-values can be calculated as:

$$P_m = \frac{\#(b : |t_{mb}| \geq |t_m|)}{B}. \tag{10}$$

Adjusting for multiple testing can be conducted by controlling the False Discovery Rate (FDR, Lin et al., 2012) or by controlling Family Wise Error Rate (FWER, Lin et al., 2012).

Benjamini and Hochberg (BH)

Suppose that there are m hypotheses to be tested with m_0 true null hypotheses and m_1 false null hypotheses. Benjamini and Hochberg defined the False Discovery Rate (FDR) as the expected proportion of false rejections among the rejected hypotheses (number of true null hypotheses that are rejected/total number of rejected

hypotheses). Let p-value of the i^{th} nanomaterial be denoted by $P_{(i)}$. In BH procedure, ordered p-values is compared to the critical value, $q.i/m$ with q as the desired level of the FDR. $H_{(i)}, ..., H_{(k)}$ will be rejected if k=max$\{i : P_{(i)} \leq q.i/m:\}$ exists.

Benjamini and Yakutieli (BY)

Benjamini and Yakutieli (BY) procedure modified the bound to be $q(\sum_{j=1}^{m} 1/j).m_0/m$ and the k to be max$\{i : P_{(i)} \leq q.i/[m.(\sum_{j=1}^{m} 1/j)]$.

Using BH or BY procedure, the p-values are adjusted into

$$\tilde{P}_{(i)} = min_{k=i,...,m} \left[min \left(\frac{m.C}{i} P_i, 1 \right) \right] \tag{11}$$

with $C = 1$ for BH procedure and $C = \sum_{j=1}^{m} 1/j$ for BY procedure (Lin et al., 2012).

References

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